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Potentiality of plant extract against *Colletotrichum gloeosporioides* causing anthracnose of Mango

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ABSTRACT

Botanicals were applied in *in vitro* against *Colletotrichum gloeosporioides* isolated from anthracnose affected mango. The study investigates to appropriate natural management. Five botanicals *viz.* korola fruit, amloki fruit, marigold leaves, neem leaves and mikania leaves extract were used and extracted 100 ml of each. By using Poison food technique 2 ml plant extract and 20 ml PDA medium containing petri plates were inoculated with 5 mm mycelial block of 3 days old pure culture of *C. gloeosporioides*. After7 days of inoculation (DAI), significantly the highest percent inhibition 58.49% was found from mikania followed by neem (46.25%), amloki (41.03%), korola (39.24%) and marigold (36.31 %) over control. Three higher concentrations (15, 20 and 25 ml) were prepared from highly performed extract was also tested. Among the concentrations 25 ml was gave significantly highest 72.33% inhibition followed by 68.15% and 64.56% at 20ml and 15ml respectively. In conclusion, with the increases concentrations percent inhibition also increases.

Keywords: Mango, anthracnose, *C. gloeosporioides,* plant extract, Concentrations

1. INTRODUCTION

Mango (*Mangifera indica* L.) is widely regarded as one of the best fruits, and it is a highly prized fruit crop in Bangladesh. India is the world's largest mango producing country, with 235348 hectares of land under cultivation and a total production of 1222368 metric tons per year, with an average yield of 5.19 per acre (BBS, 2020). However, when compared to other mango producing countries around the world, the yield is extremely low in Bangladesh (Hossain and Ahmed, 1994). India, China, Thailand, Indonesia, Pakistan, Mexico, Brazil, Bangladesh, Nigeria, and the Philippines are the top ten mango producing countries in the world (Borkar et al. 2021). Whereas India is in first place, Bangladesh is in eighth place (FAOSTAT, 2021). There is numerous diseases to blame for the low yield. Anthracnose, caused by C. *gloeosporioides*, is one of the most common of these diseases (Nelson, 2008). C. *gloeosporioides* is typically found in ripe fruits because the fungus remains dormant in fruit set during the growing season, and it causes significant damage primarily during the postharvest period (Nishijima, 1993; Ploetz, 2009). If rain falls heavily during the flowering period and fruit formation,

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it creates an ideal environment for anthracnose disease, which can cause damage to inflorescences and the drop of young fruits, resulting in significant economic losses due to production disruption. According to Islam et al., 2018, anthracnose caused losses of 17 to 36 percent of mango fruits in Bangladesh. Fungicides are frequently used to control anthracnose diseases before and after harvest. Excessive use of benomyl, thiophanate methyl and thiabendazole as sprays has led to a decrease of effectiveness in certain areas where pathogen resistance to fungicides has been reported (Spalding, 1982). Thus the use of fungicide is restricted due to community concerns over probable toxicological risks to people (Ansari, 1995). However, other ecofriendly control methods are needed because of the harmful effects of using of chemicals, become resistance to different fungicides and high rate of new chemicals. Though higher concentrations of chemicals can overcome anthracnose disease but it increases the risk of high toxic residues, which is very harmful for human being, because fresh mango fruit is consumed just after harvest. The integration of a number of practices targeting to reduce or eliminate undesirable side effects triggered by chemicals used for controlling major mango diseases is the most exact option for solving the problem (Chowdury and Rahim, 2009).

Hence, this experiment was conducted with the aim of providing broader options by evaluating the antifungal activity of botanical extracts from selected plants against C. *gloeosporioides*.

2. MATERIAL AND METHODS

Collection of sample

Anthracnose infected mangoes were collected from local market near the Patuakhali Science and Technology University (PSTU) campus (Figure 1). The experiment was carried out in the Plant Pathology Laboratory of the Department of Plant Pathology, PSTU, Bangladesh during the period of July, 2019 to March, 2020.

Figure 1. Anthracnose affected mango

Isolation and pure culture of fungi

The target fungi were isolated following 'Tissue planting' method on PDA medium. Pure culture was done by transferring three days old 5 mm mycellial block of *C. gloeosporioides* into PDA containing Petri plates and incubated for seven days in an incubator at 25°C. Then Identification of the fungi was done following standard methods.

Collection and Preparation of botanical extract

Five commonly available botanicals namely Korola, Amloci, Marigold, Neem and Mikania were collected. Plant extracts were made by grinding the 100 g washed plant parts of every plant species in 100 ml distilled water and filtered were done through filter paper Whatman No. 1 to obtained standard plant extracts (100%) (Table 1).

Table 1. List of plant Parts used against *C. gloeosporioides* of mango

Sl.	Botanical name	Scientific name	Family	Plant parts used
1	Corolla	*Momordica charantia*	Cucurbitaceae	Fruits
2	Amloci	*Phyllanthus emblica*	Euphorbiaceae	Fruits
3	Marigold	*Tagetes Erecta*	Asteraceae	Leaves
4	Neem	*Azadirachta indica*	Meleaceae	Leaves
5	Mikania	*Mikania micrantha*	Asteraceae	Leaves

Screening of plant extracts against *C. gloeosporioides*

Five crude plant extracts were *in vitro* tested for their potentiality against *C. gloeosporioides* mycelia growth using the poisoned food

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technique (Schmitz, 1930). Each of 10 ml botanical extract was mixed thoroughly with 100 ml PDA medium (autoclaved and kept for 40^{0}C) in conical flasks. PDA medium without botanical extract served as control. From the Mixer 20 ml warm PDA medium was poured aseptically in 8cm petri plates and kept for solidification. Then all the plates were inoculated by 5 mm culture block of the check fungus aseptically, from five days old pure culture of *C. gloeosporioides*. The block was placed on PDA medium containing plate in reversed position in the center of the petri plates and incubated at 25±2°C. Each treatment was replicated fifth. The diameters of fungal colonies were measured by taking average of the two diameters taken right angles for each colony when medium in the untreated control plates were fully covered by mycelia growth of the check fungus.

Calculation

Percent inhibition of mycelial growth data were calculated by using the following equation (Vincent, 1947).

$$\text{Percent inhibition} = \frac{C-T}{C} \times 100$$

Where, C is the colony diameter in control and T is treatment.

Effect of different concentration on growth inhibition of *C. gloeosporioides*

Among the five plant extracts were tested. The best performed extract was again tested with their three higher doses 15ml, 20ml and 25ml respectively. For each concentration, the experiments were performed in five replicates. Data were collected and percent inhibitions of mycelial growth in treated plates were calculated by applying the formula given by Vincent (1947) as earlier I described.

Analysis of data

All the collected data were to analyze by using Minitab software version 17 with 5 replicates and means were compared Tukeys method at 95% confidence level.

3. RESULTS AND DISCUSSION

In vitro* evaluation of selected botanicals against *C. gloeosporioides

Effect of the botanicals in controlling anthracnose of mango (*C. gloeosporioides*) was evaluated in *in vitro* condition.

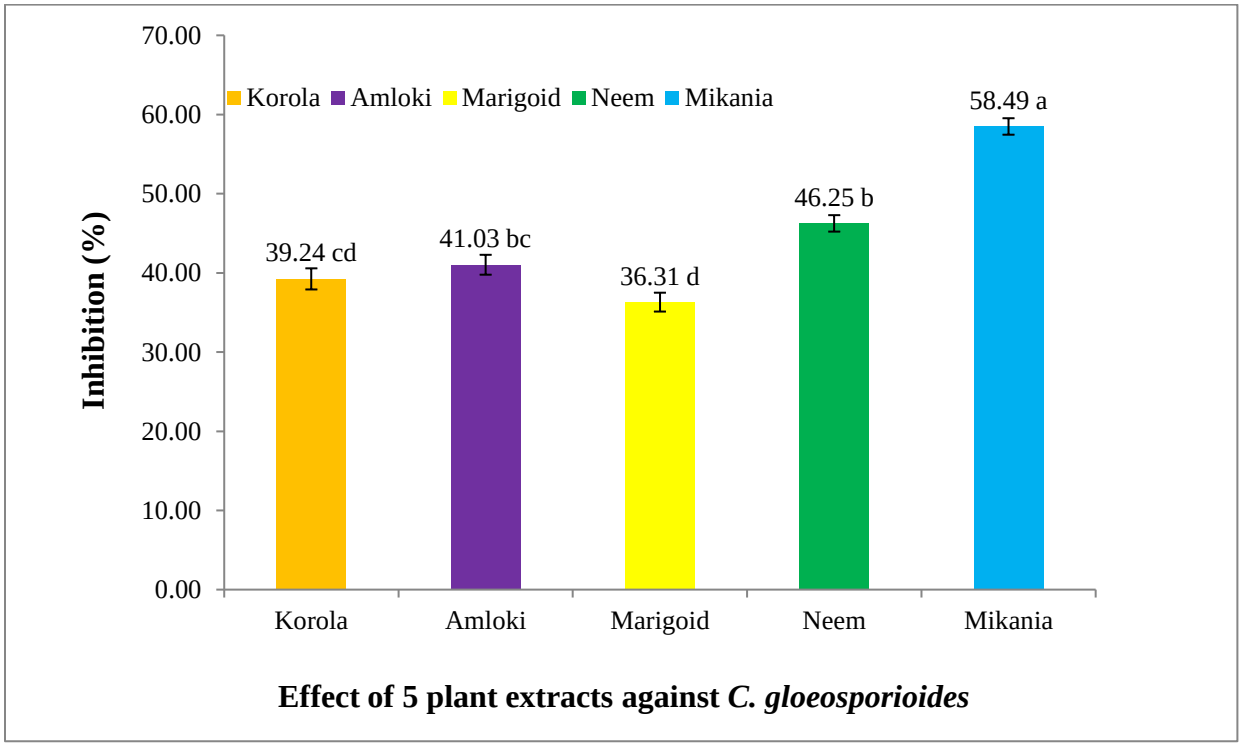

Figure 2. Graph showing the effect of plant extract on mycelial growth of *C. gloeosporioides*

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Treated 20 ml PDA medium containing petriplates were inoculated by 5 mm mycellial block of *C. gloeosporioides* at the centre of plates. Though all plant extracts exhibited different levels of antifungal activities against *C. gloeosporioides* mycelia Corolla, Amloci, Marigold, Neem did not effectively prevent mycelia growth. Interestingly the Mikania leaf extract exhibited the higest inhibition activities against *C. gloeosporioides* mycellial growth (58.497%) followed by Neem (46.25%), Amloci (41.03%), Corolla (39.40%), Marigold (36.71%) (Figure 2 and 3).

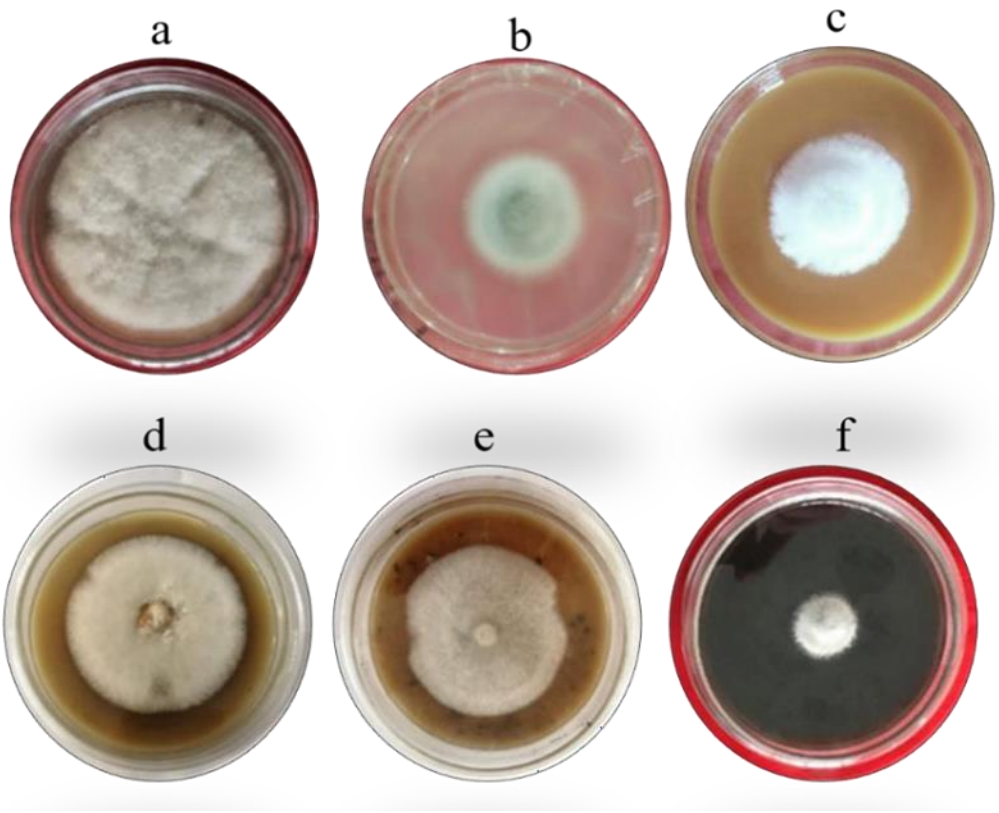

Figure 3. Screening of Botanicals for inhibiting mycelial growth of *C. gloeosporioides* a) Control-full growth ofmycelium b) Korola, c) Amloki, d) Marigold, e) Neem, and f) Mikania restricted the mycelial growth respectively.

Effect of different concentrations on growth inhibition of *C. gloeosporioides*

The highly performed mikania leaf extract were again tested against *C. gloeosporioides* by preparing their three different concentrations (15, 20, 25 ml). The highest percentage inhibition 72.33% was observed at 25% concentration and the inhibition percentage 68.15% was observed at 20% concentration which was statistically similar followed by 64.56% at 15% concentration. With the increase of concentrations, the mycelial growth inhibitions wereincreased significantly (Figure 4 and 5).

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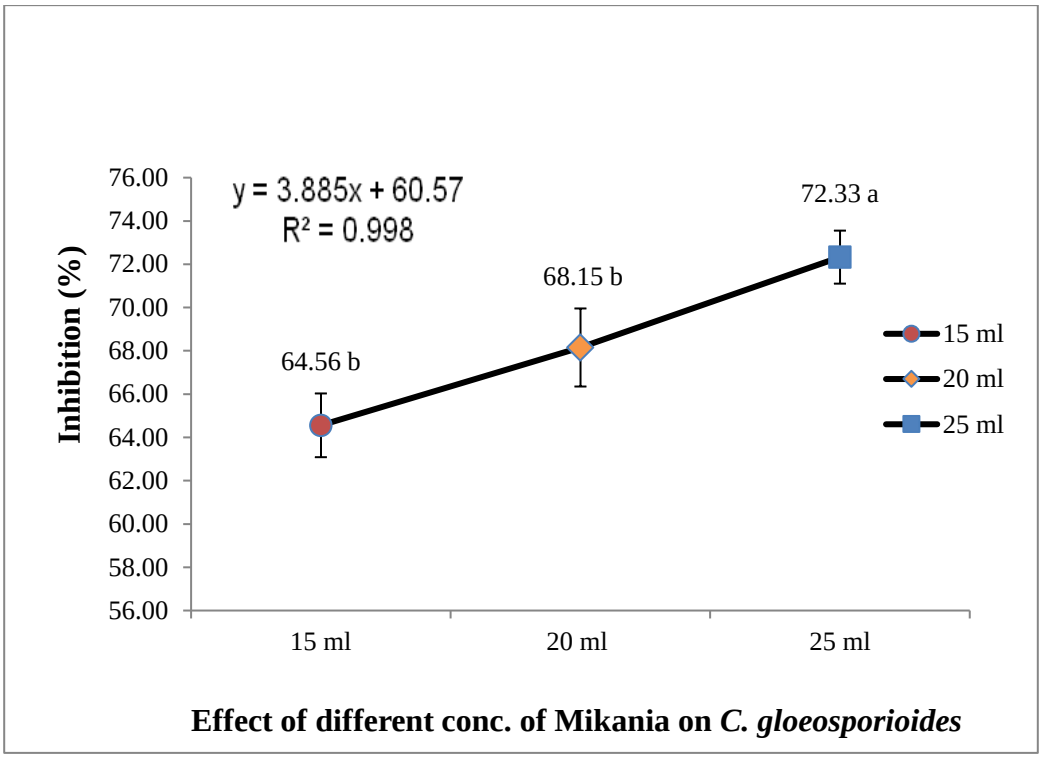

Figure 4. Graph showing effect of different concentration on growth inhibition of *C.gloeosporioides*

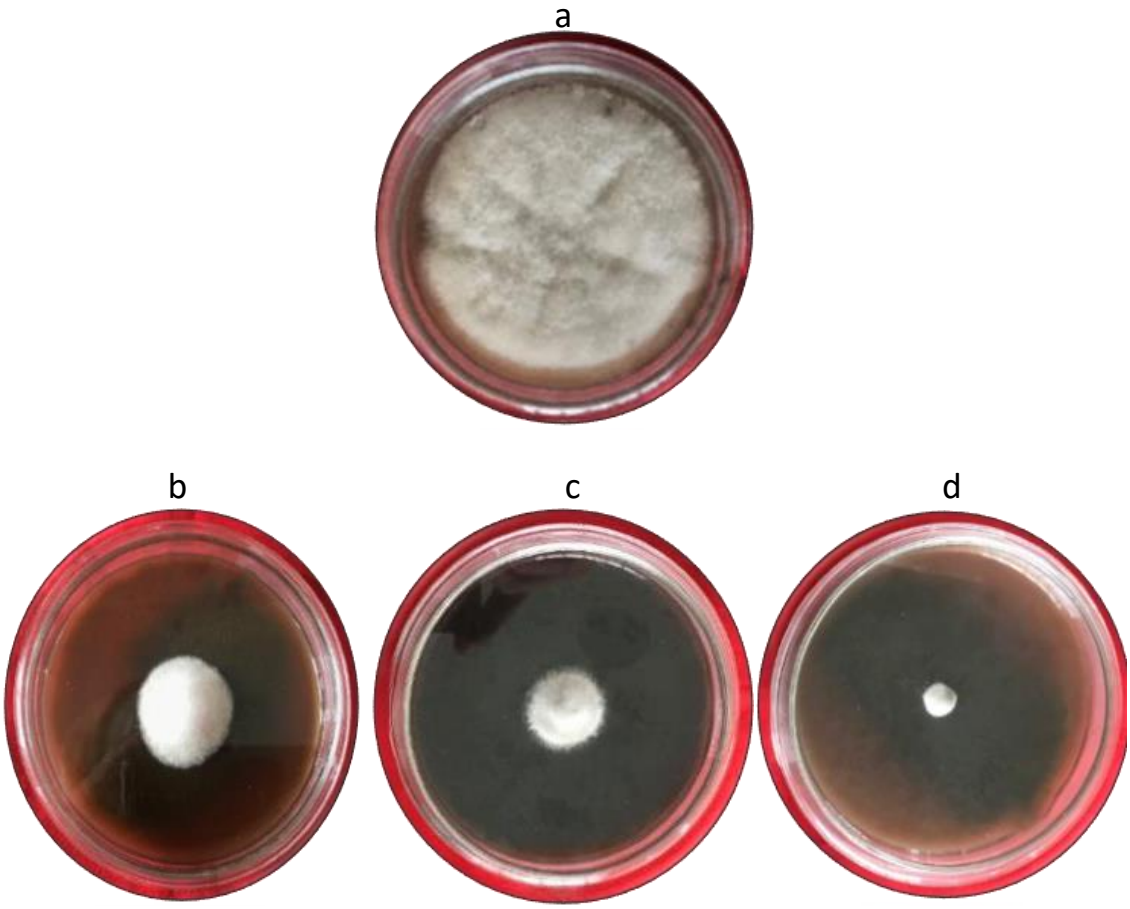

Figure 5. Different concentration of Mikania inhibits growth of *C. gloeosporioides*
a) Control-full growth of mycelium b) 15 ml, c) 20 ml and d) 25 ml inhibited the mycelialgrowth respectively.

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4. CONCLUSION

In conclusion, the study shows that mikania leaves extract have strong antifungal activities against *C. gloeosporioides.* This may suggest their potentiality for controlling anthracnose diseases of mango. More extensive study and *in vivo* efficacy remains to be determined.

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Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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